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128850

MRID No. 413961-06

DATA EVALUATION RECORD

1. **CHEMICAL:** Glufosinate.
Shaughnessey No. 128850.

2. **TEST MATERIAL:** HOE 039866 Technical; ammonium-DL-homoalanin-4-yl(methyl)phosphinate; Code #HOE 039866 OH ZC96 0002; Lot No. 22.01.87; 96.3% active ingredient; a white powder.

3. **STUDY TYPE:** Mollusc 48-hour Embryo-Larval Study. Species Tested: Quahog clam (Mercenaria mercenaria).

4. **CITATION:** Surprenant, D.C. 1988. Acute Toxicity of HOE 039866 Technical Substance (Code: HOE 039866 OH ZC96 0002) to Embryos and Larvae of the Quahog Clam (Mercenaria mercenaria). Prepared by Springborn Life Sciences, Inc., Wareham, Massachusetts. Report No. 87-12-2587. Study No. 1719.0487.6107.514. Submitted by Hoechst Celanese Corporation, Somerville, New Jersey. MRID No. 413961-06.

5. **REVIEWED BY:**

Kimberly Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Kimberley Rhodes
Date: June 1, 1990

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 6/1/90

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: R.C. Petrie M. Reynolds 12/24/90
Date: 12/20/90

7. **CONCLUSIONS:** This study appears scientifically sound and fulfills the Guideline requirements for a Quahog clam embryo-larval test. The 48-hour EC50, based upon nominal concentrations, of HOE 039866 to Quahog clams (Mercenaria mercenaria) was determined to be >125 mg/L, the highest concentration tested. Therefore, HOE 039866 is classified as practically non-toxic to quahog clams. The NOEC was determined to be 75 mg/L after 48 hours.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Embryos of the quahog clam (Mercenaria mercenaria) were obtained by induced spawning of sexually mature adult quahogs at a commercial shellfish hatchery located in Cape Cod, Massachusetts. Adults had been maintained in the hatchery in natural seawater with a typical salinity range of 28-32 parts per thousand (ppt).

Sexually mature quahogs were induced to spawn by placing them in individual containers of seawater which were placed in a heated water bath at 24°C. The water temperature in the containers was raised over a 5-minute period to approximately 30°C in the presence of viable sperm excised from the gonad of a sexually mature male quahog. Fertilization was achieved by adding a controlled amount of sperm to eggs released into the spawning chambers and was confirmed microscopically. Density of the embryos in the inoculum solution was determined by a Sedgwick-Rafter/Whipple disk count using 1 mL of the embryo suspension from the spawning chamber.

B. **Test System:** The test was performed in 1.0-L glass beakers containing 900 mL of test solution. All exposure levels were triplicated and the control was quadruplicated. The test vessels were maintained at 20-21°C under a photoperiod of 16 hours of light and 8 hours of darkness for the 48-hour exposure period.

The dilution water was natural filtered seawater collected from the Cape Cod Canal, Bourne, Massachusetts. The water was filtered through a 5-µm core filter. The dilution water control was characterized as having a dissolved oxygen concentration of 7.4 mg/L, a pH of 7.9, and a salinity of 32 ppt at test initiation.

C. **Dosage:** Mollusc 48-hour embryo-larval static test.

D. **Design:** A control and five nominal HOE 039866 concentrations of 16, 27, 45, 75, and 125 mg/L were tested. Each exposure and control vessel was inoculated with approximately 28,616 embryos within 2 hours after fertilization. After 48 hours, the larvae from each chamber were collected in a 37-μm mesh size sieve, rinsed into a plastic bottle with 19 mL of filtered seawater and preserved with 1 mL of neutralized formalin. The number of normally developed 48-hour-old larvae was determined microscopically by a Sedgwick-Rafter/Whipple disk count from each test and control container.

The dissolved oxygen, pH and temperature of the test solutions were measured at 0 and 48 hours of the exposure period. Dissolved oxygen concentrations and pH's were measured in the 3-liter volume of each test solution at test initiation and in the composited replicate solutions after the larvae were removed at test termination. The temperature was also continuously measured in a control vessel during the exposure.

E. **Statistics:** Results of the toxicity test were used to calculate the percentage reduction of normal quahog clam larvae from each test concentration when compared to the control. The percentage reduction of normal 48-hour embryos was determined as follows:

$$\% \text{ Reduction} = \frac{\text{mean \# of normal control larvae minus mean \# of normal exposed larvae}}{\text{mean \# of normal control larvae}} \times 100$$

The biological results derived from the 48-hour test are used to statistically estimate a median effect concentration (EC50) and the 95% confidence interval. The EC50 is the estimated concentration of test material in seawater which reduce normal embryo/larval development of exposed quahogs by 50 percent of the response observed for the control quahogs. The reduction of normally developed larvae is calculated as the ratio of the mean number of larvae of exposed clams to the mean number of control larvae. The EC50 value is empirically estimated, if the biological response in a test precludes linear regression analysis of the data. If the test data indicate the EC50 to be greater than 100 mg/L of the test material for clam embryos-larvae, empirical estimation is used.

The no-observed-effect concentration (NOEC) is determined by subjecting the biological response data for all treatment levels and controls to analysis of variance. William's Test is used to determine the highest treatment level not significantly different ($P \leq 0.05$) than the control, which is identified as the NOEC.

12. **REPORTED RESULTS:** The nominal test concentrations of HOE 039866 and the corresponding effects on clam embryo-larval development observed in this test are presented in Table 2 (attached). Exposure to 125 mg/L HOE 039866 reduced the number of normally developed clam larvae by 31% when compared to the control development. Analysis of variance and William's Test indicated this response to be significantly ($P \leq 0.05$) different than the control. Development of clam embryos-larvae exposed to test concentrations $\leq$ 75 mg/L were not significantly different than the control, establishing 75 mg/L as the no-observed-effect concentration.

The 48-hour EC50 was empirically estimated to be > 125 mg/L HOE 039866, the highest concentration tested. The no-observed-effect concentration was 75 mg/L. Based on criteria established by EPA (1985) HOE 039866 is classified as practically non-toxic to clam embryo-larvae.

Water quality was unaffected by the concentrations of HOE 039866 tested and was satisfactory for the normal development of clam embryos and larvae. The salinity was 32 ppt and the temperature range was 20-21°C. No surface film or undissolved material was observed, indicating the EC50 is a reliable estimate of the acute toxicity of the test material solubilized in seawater to quahog embryos and larvae.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the author.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with the FIFRA Good Laboratory Practice Standards.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP and ASTM as follows:

o According to the ASTM, non-viable (heat-killed) sperm should be used to induce female quahogs to spawn. In this test, viable sperm were used.

o The SEP states that embryos should be tested within one hour of spawning and after fertilization. This test used embryos 2 hours after fertilization.

o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether 15- to 30-minute transition periods between light and dark were maintained.

B. Statistical Analysis: Statistical analysis was not needed to calculate an EC50 value since the highest test concentration (125 mg/L) resulted in only 31 percent reduction of normally developed clam larvae when compared to the control. Therefore, the 48-hour EC50 value was determined to be >125 mg/L, the highest nominal concentration tested.

The reviewer evaluated the no-observed effect concentration (NOEC) by using analysis of variance (ANOVA). The reviewer found no significant difference between the control and each HOE 039866 concentration. However, the author found a significant difference between the control and the highest nominal test concentration (75 mg/L). Therefore, based on the author's results, the NOEC was determined to be 75 mg/L nominal concentration.

C. Discussion/Results: The study results appear to be scientifically valid. The EC50 value, based on percentage reduction of normal quahog clam larvae after 48-hour of exposure to HOE 039866, was >125 mg/L nominal concentration. Therefore, HOE 039866 is classified as practically non-toxic to quahog clams (Mercenaria mercenaria). The NOEC was determined to be 75 mg/L nominal concentration.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 05-16-90.

Shaughnessey No. 128850 Chemical Name Glufosinate (HOE-039866) Chemical Class ____ Page ____ of ____

Study/Species/Lab/Accession | Chemical % a.i. | Results | Reviewer/Date | Valid Study

14-Day Single Dose Oral LD_{50}

Species ____

Lab

Acc.

LD50 = . mg/kg (95% C.L.) Contr. Mort.(%) =

Slope = # Animals/Level = Age(Days) = Sex =

14-Day Dose Level mg/kg/(% Mortality)

( ), ( ), ( ), ( ), ( )

Comments:

14-Day Single Dose Oral LD_{50}

Species ____

Lab

Acc.

LD50 = mg/kg. (95% C.L.) Contr. Mort.(%) =

Slope = # Animals/Level = Age(Days) = Sex =

14-Day Dose Level mg/kg/(% Mortality)

( ), ( ), ( ), ( ), ( )

Comments:

8-Day Dietary LC_{50}

Species

Lab ____

Acc.

LC50 = ppm (95% C.L.) Contr. Mort.(%) =

Slope = # Animals/Level = Age(Days) = Sex =

8-Day Dose Level ppm/(%Mortality)

( ), ( ), ( ), ( ), ( )

Comments:

8-Day Dietary LC_{50}

Species

Lab ____

Acc.

LC50 = ppm (95% C.L.) Contr. Mort.(%) =

Slope = # Animals/Level = Age(Days) = Sex =

8-Day Dose Level ppm/(%Mortality)

( ), ( ), ( ), ( ), ( )

Comments:

48-Hour EC_{50}

Species Mercenaria mercenaria

Lab Springborn Life Sciences, Inc. 96.3%

Acc. 413961-06

EC50 = 7125 ppm (95% C.L. N/A) Reduction Contr. Mort.(%) = N/A

Slope = N/A # Animals/Level = 28,616 Sol. Contr. Mort.(%) = N/A

% Reduction Temperature = 20-21°C 5/16/90 Core

48-Hour Dose Level ppm/(%Mortality)

16.1 (0), 271 (6), 451 (18), 75 (21), 125 (3)

Comments: Based on nominal concentrations

96-Hour LC_{50}

Species

Lab ____

Acc.

LC50 = pp__ (95% C.L.) Con. Mor.(%) =

Slope = # Animals/Level = Sol. Con. Mor.(%) =

Temp. =

96-Hour Dose Level pp /(%Mortality)

( ), ( ), ( ), ( ), ( )

Comments:

96-Hour LC_{50}

Species

Lab ____

Acc.

LC50 = pp__ (95% C.L.) Con. Mort.(%) =

Slope = # Animals/Level = Sol. Con. Mort.(%) =

Temp. =

96-Hour Dose Level pp /(%Mortality)

( ), ( ), ( ), ( ), ( )

Comments:

Table 2. Toxicity of HOE 039866 OH ZC96 0002 to embryos-larvae of quahogs (Mercenaria mercenaria) exposed for 48 hours in static, filtered seawater.

Nominal concentration (mg/L)	48-Hour Number of normal larvae Mean	SD[a]	48-Hour Reduction of normal 48-hour larvae (%)
125	14,867	2,532	31[b]
75	17,000	2,905	21
45	17,667	5,601	18
27	20,200	1,800	6
16	21,533	1,701	0
Control	21,600	4,283	NA

[a] Standard deviation.

[b] Significantly different ($P \leq 0.05$) than control.

Analysis of Variance File: hoeclam Date: 06-01-1990

FILTER: None

N's, means and standard deviations based on dependent variable: RESPONSE

* Indicates statistics are collapsed over this factor

Factors: C nominal Concentration (mg/L)	N	Mean	S.D.
*	19	18957.8945	3936.4841
1 Control	4	21600.0000	4283.3008
2 14	3	21533.3340	1700.9801
3 27	3	20200.0000	1800.0000
4 45	3	17666.6660	5601.1904
5 75	3	17000.0000	2905.1677
6 125	3	14866.6670	2532.4561

Fmax for testing homogeneity of between subjects variances: 10.84
Number of variances= 6 df per variance= 2.

Analysis of Variance Dependent variable: RESPONSE

Source	df	SS (H)	MSS	F	P
Between Subjects	18	278926340.0000			
C (CONC)	5	119166320.0000	23833264.0000	1.939	0.1542
Subj w Groups	13	159760016.0000	12289232.0000		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	21600.000	6	14866.667
2	21533.334		
3	20200.000		
4	17666.666		
5	17000.000		

Comparison	Bon-ferroni	Dunnett
1 > 2		
1 > 3		
1 > 4		
1 > 5		
1 > 6		
2 > 3		N.A.
2 > 4		N.A.
2 > 5		N.A.
2 > 6		N.A.
3 > 4		N.A.
3 > 5		N.A.
3 > 6		N.A.
4 > 5		N.A.
4 > 6		N.A.
5 > 6		N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).